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Glutamine Synthase Expression Profiling and Selective Inhibitor for *Burkholderia psuedomallei* K96243: An *In-silico* Approach

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Abstract

Melioidosis is a potentially fatal infection caused by the Gram-negative bacillus, Burkholderia pseudomallei following an encounter with contaminated soil or surface water. This study was to identify the highly expressed gene in Burkholderia pseudomallei and model the target protein's three-dimensional structure. Further, the study was intended to determine the better binding candidates from the existing drugs against the target protein for melioidosis. The highly expressed gene was identified by KEGG pathway and their target protein was modeled using homology modeling. The modeled structure was validated by PROCHEK and it can be further used for docking studies against existing antibacterial drugs using Argus Lab. The ADME properties of drugs were analyzed by the ADME/Tox WEB. The results revealed that, glutamine synthetase was highly expressed in Burkholderia pseudomallei and its 3D model was generated. The structure validation revealed that, the structure was reliable and reasonable. The docking studies revealed that the Chloramphenicol compound has higher docking score against the protein glutamine synthetase compared to other existing compounds. The acceptable range of the ADME and biological activity prediction of Chloramphenicol compound depicts better pharmacological properties and possible drug-likeliness. This study concluded that, the compound Chloramphenicol may be a better inhibitor for glutamine synthetase protein. This compound may lead to development of effective drug against Melioidosis.

Keywords: *Burkholderia pseudomallei, Docking and ADME Properties, Glutamine Synthetase, Homology Modeling.*

Introduction

Melioidosis, an infectious disease prevalent throughout Southeast Asia and Australia, is primarily spread through soil contact [1]. *Burkholderia pseudomallei*, a gram-negative β-proteobacterium, primarily residing in soil surfaces during the rainy season in tropical and subtropical regions around the world is the causative agent of melioidosis [2]. It is classified by the Centers for Disease Control (CDC) as a Tier 1 select agent because of its potential for aerosolization and illicit use as a bioweapon [3]. This highly pathogenic bacterium can cause a wide range of symptoms, from mild localized infections to severe sepsis and death. Immunocompromised individuals, particularly those with diabetes or chronic alcohol consumption, are especially vulnerable to infection by this bacterium [4]. Melioidosis predominantly spreads during the monsoon season and can occur after soil exposure, even

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without physical injuries or wounds [5]. However, human-to-animal and person-to-person transmission routes are also feasible in rare instances [6]. As mentioned earlier, individuals with diabetes mellitus are frequently diagnosed with melioidosis, making it an opportunistic infection. These patients have a twelve-fold increased risk of contracting the disease [7]. Statistically, it has been estimated that the incidence rates in North East Thailand and Northern Australia reach up to 50 cases per 100,000 persons. Unfortunately, North East Thailand is distinctive with a fatality rate of 40%. Due to a lack of knowledge about the disease, misdiagnosis, and unwillingness to seek medical aid, the mortality rate as well as the prevalence of illnesses and deaths has not been adequately reported [8]. A 2016 modeling study predicted that there would be 165,000 cases and 89,000 fatalities globally each year. 44% of this burden is accounted by South Asian countries. Based on data from the Global Burden of Disease Study 2019, this incidence and mortality are linked to six main risk factors: raised systolic blood pressure, tobacco use, dietary hazards, high fasting plasma glucose, increased BMI, and malnutrition. According to several researches, diabetes mellitus is the most prevalent risk factor for the condition [9]. Although the disease has few to no symptoms for an early diagnosis and its epidemiology, a few genetic technologies, such as whole genome sequencing and multilocus sequence typing, provide knowledge about the disease's genetic link and epidemiology [10].

B. pseudomallei possesses strong adaptability in a broad spectrum of environmental conditions. Nevertheless, there is a paucity of data about the organism's unique genetic makeup with merely 69 among the 6,817 isolates in PubMLST related to Africa. Therefore, comprehension of the genealogy associated with disease aggravation might aid in disease management [11,12]. Indeed, antibiotics are the keystone of melioidosis therapy, which comprises intravenous ceftazidime or meropenem and is accompanied by adjunctive therapies. However, pathogens have become resistant to antibiotics, constraining the therapeutic options for melioidosis, eventually causing treatment failure and significant fatalities [13]. Therefore, the accession of *B. pseudomallei's* full genome sequence and annotations might facilitate the prediction of new targets to maximize therapeutic choices. Our study emphasizes the molecular and cellular basis of pathogenesis in melioidosis with a comprehensive overview of the current knowledge on how *B. pseudomallei* can cause the disease. Gene expression profiling facilitated the identification of target protein glutamine synthetase (glnA). The three-dimensional structural models were eventually built using homology modeling tools. Prospective *B. pseudomallei* inhibitor leads were screened using molecular docking analysis based on the docking scores. The best fit compounds would be indispensable in the development of an effective antibacterial medication for Melioidosis.

Materials and Methods

Kyoto Encyclopedia of Genes and Genomes (KEGG) Database

Kyoto Encyclopedia of Genes and Genomes (KEGG) database is a knowledge base for systematic analysis of gene functions in terms of the networks of genes and molecules. The major component of KEGG is the pathways database that consists of graphical diagrams of biochemical pathways including most of the known metabolic pathways [14]. The amino acid metabolism (Entry No-bps00250, alanine, aspartate, and glutamate metabolism) pathway of *Burkholderia pseudomallei* K96243 was analyzed using the KEGG pathway database and the expressed genes were collected for further studies.

Java Codon Adaptation Tool (JCat)

The innovative method for the variation of target gene codon usage to mainly sequenced

prokaryotes and selected eukaryotic gene expression groups was expanded to recover the heterologous protein production. In contrast to existing tools, JCat does not require the manual description of vastly expressed genes. Thus, it is a very rapid and easy method [15]. The list of the genes collected from KEGG pathway along with its sequences in FASTA-format were uploaded in JCat tool to calculate Codon Adaptation Index (CAI) values. The highly expressed gene was identified based on the CAI values.

Homology Modeling

Three-dimensional protein constructions offer valuable insights into the molecular basis of protein function and serve as the bedrock for an effective design of experiments such as site-directed mutagenesis, studies of disease-related mutations and the structure-based design of specific inhibitors [16].The 3D structure of Glutamine synthetase was modeled based on its structural similarity with Glutamine synthetase *Salmonella typhymurium* (PDB ID: 1F2E) by comparative protein modeling methods using optimized mode of SWISS-MODEL.

Structure Validation

Modeled structure of glutamine synthetase was subjected to a series of tests for its internal consistency and the main geometric parameters of the model were determined by PROCHECK. Backbone conformation was evaluated by the inspection of the psi/phi anglesin Ramachandran plot analysis, and the over-all quality factor was analyzed by Verify 3-D.

Active Site Prediction

The binding site of the modeled Glutamine synthetase protein was identified by Q- Site Finder, which relatively reliable method of predicting the ligand binding site in the target protein. Q-Site Finder predicts the binding site by binding hydrophobic (CH3) probes to the protein and finding clusters of probes with the most favorable binding energy. These clusters are placed in rank order of the likelihood of being a probable binding site according to the total binding energies for each cluster [17].

Ligand Selection

The antibacterial ligands were collected from the drug bank database which is currently being used for the treatment of melioidosis. These ligands were prepared for further docking studies with the target protein of glutamine synthetase.

Molecular Docking

The docking studies were carried out by Argus Lab Version 4.01 software and the procedure was followed by previously described studies with slight modifications [18]. The atoms of the prepared ligands were then set to the appropriate hybridizations (sp3, sp2, etc) followed by the addition of the hydrogen atoms and the geometry of each compound were optimized. A maximum number of 500 poses was set to increase the bindingprecision and spacing between the grid points was set at 0.4 Å. Poses were rank-ordered by docking energy and the pose with the lowest energy was chosen as the predicted receptor- bound conformation of the ligand. The binding energies of the ligand were calculated with these parameters.

ADME / Tox WEB

The ADME Suite of predictors provides essential desktop software modules for the calculation of properties relating to absorption, distribution, metabolism, and excretion. The various models were built using a combination of statistics, expert knowledge, and scientific intuitions [19]. These tools provide vital information that allows scientists to better understand the molecular structures they work with, and guide ongoing research. ADME and toxicity properties of the ligand molecule were checked by ADME / Tox WEB.

Results

KEGG Pathway Database

The KEGG pathway revealed that, in *Burkholderia psuedomalle* K96243 organism, 35 genes were expressed in alanine, aspartate and glutamate metabolism.

JCat Tool

JCat tool offers the possibility to adapt the codon usage of a gene of interest to one of the selected organisms. For this purpose, the CAI values for a list of genes were calculated. Based on the CAI values, JCat tool revealed that out of 35 genes, the glnA gene was highly expressed in alanine, aspartate and glutamate metabolism pathways.

Molecular Modeling

The absence of a three-dimensional structure of Glutamine synthetase motivated us to construct this model. Accordingly, the crystal structure of Glutamine synthetase of *Salmonella typhymurium* (PDB ID: 1F2E) was selected as an appropriate template for modellingand the 3D structure of Glutamine synthetase was generated shown in Figure 1 by SWISS-MODEL based on comparative template model structure.

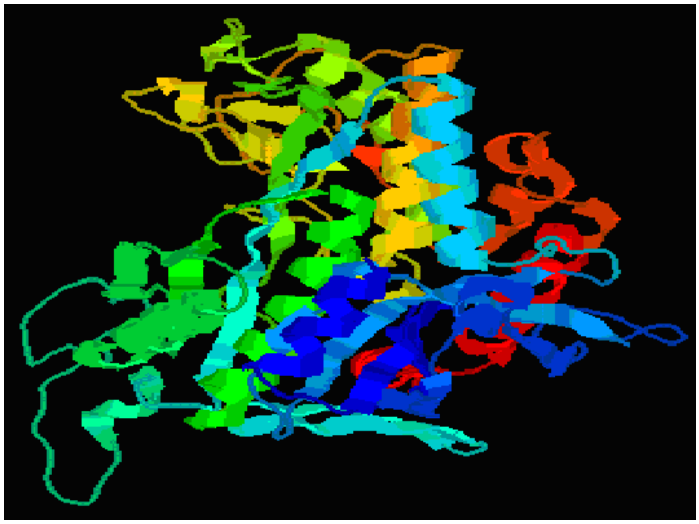

Figure 1. Three-Dimensional Modelled Structure of Glutamine Synthetase Protein

The backbone conformation of the refined model was validated by Ramachandran plot obtained through PROCHECK. The generated model was found to be highly plausible, only 0.3% of residues were found to span the disallowed region of Ramachandran plot, as shownin Figure 2. The overall quality factor of the computed model was 89.57%.

Q-Site Finder

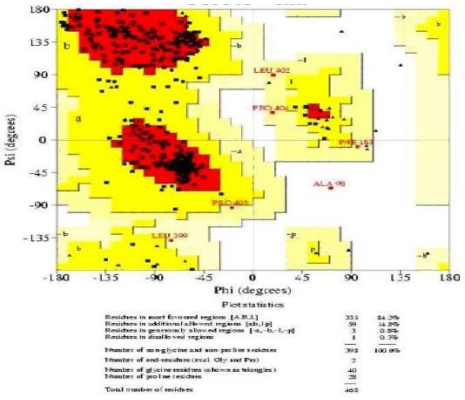

Figure 2. Ramachandran Plot of Modelled Glutamine Synthetase Protein

The Q-Site Finder produced the top ten ranked binding sites. The higher activity site was taken as the most favourable site to dock the ligands**.** The binding site of the modelled Glutamine synthetase protein is illustrated in Figure 3.

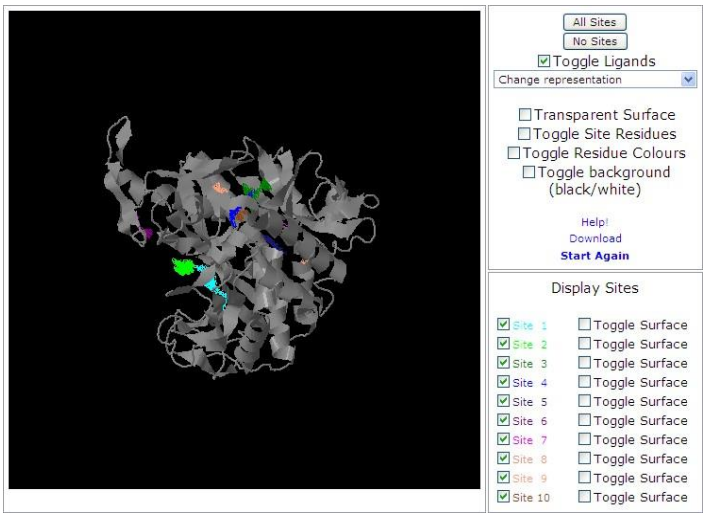

Figure 3. Predicted Active Site of the Modeled Glutamine Synthetase Protein

Docking Studies

Our molecular docking analysis investigated the potential binding of the existing anti-bacterial compounds Cefotaxime, Chloramphenicol, Clavulanate, Sulfamethoxazole, Ceftriaxone and Trimethoprim obtained from the drug bank against glutamine synthetase. Their drug category, molecular formula and chemical structure are shown in Table 1.

Table 1. List of Anti-bacterial Compounds Retrieved from the Drug Bank Database for Molecular Docking Studies with the Target Protein of Glutamine Synthetase.

Drug name	Drug type	Drug Category	Formula	Chemical Structure
Cefotaxime	Approved Small Molecule	Anti-Bacterial Agents Cephalosporins	$C_{16}H_{17}N_5O_7S_2$	
Chloramphenicol	Approved Small Molecule	Anti-Bacterial Agents Protein Synthesis Inhibitors	$C_{11}H_{12}Cl_2N_2O_5$	
Clavulanate	-	Anti-Bacterial Agents Enzyme Inhibitors	$C_8H_9NO_5$	
Sulfamethoxazole	Approved Small Molecule	Anti-Infective Agents Anti-Infectives Sulfonamides	$C_{10}H_{11}N_3O_3S$	

Ceftriaxone	Approved Small Molecule	Anti-bacterial agents Cephalosporins	$C_{18}H_{18}N_8O_7S_3$	
Trimethoprim	Approved Small Molecule	Anti-infective agents urinary Anti-infectives Antimalarials Folic Acid Antagonists	$C_{14}H_{18}N_4O_3$	

The Argus Lab docking studies revealed that Glutamine synthetase has favored the binding of the Chloramphenicol compound (Figure 4).

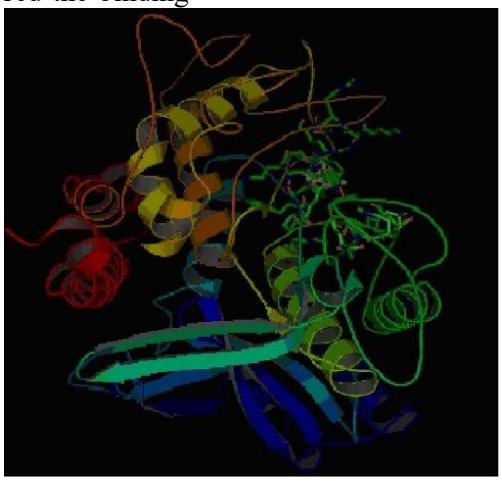

Figure 4. The Complex Structure of Glutamine Synthetase Protein and Chloramphenicol Compound

This is evident in the lowest dock score of -9.97 cal/mol among other counterparts. The favourable binding of the Chloramphenicol within the binding site of the Glutamine synthetase depicts that this compound has higher affinity and better interaction with target protein. *The docking* results are shown in Table 2.

Table 2. Docking Scores of Antibacterial Drugs Complexes with the Target Protein of Glutamine Synthetase.

Drug Name	**Docking score (kcal/mol)**
Cefotaxime	-8.6731
Chloramphenicol	-9.97697
Clavulanate	-6.51728
Sulfamethoxazole	-9.1041
Ceftriaxone	-8.8883
Trimethoprim	-3.1287

ADME Properties

The pharmacokinetic properties of these compounds are in the acceptable range and results are shown in Table 3.

Table 3. Predicted ADME and Toxicity Profiles of the Compounds using ADME/Tox WEB.

S No	Drug name	Stability (PH<2)	Oral availability (Human)	Absorption Rate Min^{-1}	Volume Distribution (L/Kg	Solubility (In water)	Protein Binding	pKa	Log P
1	Cefotaxime	Stable	<30%	0.000	0.23	insoluble	44.34%	Acid:2.50+ / - 0.50 Base: 3.50 +/- 0.50	0.10
2	Chloramphenicol	Stable	>70%	0.023	1.19	Slightly soluble	42.27%	Acid: No acid pKa Base: No base pKa	0.82
3	Clavulanate	Stable	<30%	0.000	0.22	Soluble	40.19%	Acid: 1.50 + / -0.80 Base: No base pKa	-0.31
4	Sulfamethoxazole	Stable	>70%	0.004	0.19	Soluble	85.78%	Acid:5.70 + / -0.50 Base:1.80 +/- 0.50	0.86
5	Ceftriaxone	Stable	<30%	0.000	0.25	Insoluble	71.80%	Acid: 2.50 + / -0.50 Base: 7.90 +/ -1.70	-0.46
6	Trimethoprim	Stable	>70%	0.008	1.65	Insoluble	34.55%	Acid: No acid pKa Base: 7.30 +/ -0.50	0.96

Discussion

Comprehending the diversity of species can unveil unique genomic traits and functions. Under these circumstances, natural isolates have evolved into progressively useful weapons for exploring the dependability of research findings [20,21,22]. Hence, our study provides a comprehensive approach to identify key regulators driving the molecular metabolism of *B. psuedomalle* K96243 and pathogenetic rationales in melioidosis. In addition, it focuses on screening commercially existing drugs as effective inhibitors for antagonizing the target protein for the development of antimicrobial drug to combat Melioidosis. The Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) is a popular database for integrating the complex functions and features of biological systems [23]. The data obtained from KEGG database revealed the expression of 35 genes in alanine, aspartate, and glutamate metabolism of *Burkholderia psuedomalle* K96243 organism. Further, the data were subjected to JCat server to optimize the codon sequence including elimination of unrelated restriction sites and prevention of transcription terminator. The CAI values from JCat revealed high expression of glnA gene in alanine, aspartate, and glutamate metabolism. Thereafter, homology modeling was utilized to construct reliable three-dimensional structural models for query proteins, to further rationalize experimental observations using molecular

docking analysis [24]. In this study, a homology model of *Burkholderia pseudomallei* Glutamine synthetase was developed by high structural similarity of Glutamine synthetase protein. Structural validation was carried out for the modeled structure, which shows that the model is reliable and this stable structure is further used for receptor-ligand interaction analysis. Q-Site Finder was used for mapping the binding sites of Glutamine synthetase. The residues in the binding pocket of Glutamine synthetase predicted by Q-Site finder were further validated during grid generation in the centroid of these residues for molecular docking. Molecular docking is a computational approach for prospection of binding affinity of target protein and ligands [25, 26]. Commercial antibacterial compounds were retrieved from drug bank to perform the docking studies. The molecular docking analysis revealed that among the screened drugs, 6 hit compounds were identified. The Argus Lab dock score revealed that, out of 6 hit compounds, Chloramphenicol had good binding affinity with the target protein, which was elucidated by the highly negative docking score of the compound. Based on these results, this study concluded that the Chloramphenicol compound may be a good inhibitor against the Glutamine synthetase protein. Further, ADMET profiling was performed for the 6 hit compounds. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties play versatile roles in the development of industrial drugs [27]. The ADME and biological activity prediction of this compound was under the admissible range.

Conclusion

Based on these results, this study concluded that the Chloramphenicol compound may be a good inhibitor against the Glutamine synthetase protein. Thus, this compound might aid for the development of drug candidate against *Melioidosis.*

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Conflict of Interest Statement

We declare no conflict of interest.

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